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OVERRIPENESS OF THE EGGS AS CAUSAL FACTOR IN
THE APPEARANCE OF MULTIPLE FORMATIONS AND TERATISMS

E. Witschi

ABSTRACT. Both heat and overripeness of eggs are seen to
be a factor in producing multiple formations and teratisms
in frogs. Radical malformations were observed to result
from either factor. Limiting himself to a description of
of observations of the living object, the author
investigates the causal genesis of these phenomena. The
following two causes are specifically named: loosening
of intercellular bonds and varying degree of damage of
different parts of the egg resulting from overripeness.

I reported in the *Festschrift* for Friedrich Zschokke (1920) on some multi- /33*
armed frogs that I had obtained accidentally during experimental work [1].
They appeared in a culture of overripe eggs that had been kept at maximum
temperature. It remained undetermined, whether the result should be ascribed
primarily to one or the other of the two experimental factors. On the other
hand there was no so-called germinal variation involved; i.e., no character-
istic inherited from ancestors, because among the numerous siblings that were
raised at normal temperature no corresponding multiple formations occurred.

In order to settle the still open alternative, I experimented again in
the spring of 1921 with overripe frog eggs, but eliminated the heat factor.
I let some of the eggs and embryos develop at optimum temperature, i.e. bet-
ween 16-21° C. (Culture H_{20}). Normally, however, under natural conditions
the temperature is below 12°. For that reason I also kept a parallel cold
series (Culture H_{11}).

Multiple formations resulted from both cultures, but the percentage was
much higher in the heat than in the cold.

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* Numbers in the margin indicate pagination in the foreign text.

Corresponding to the high degree of overripeness chosen, the malformations this time were of a multifarious nature. Multi-armed frogs occurred again, so that the overripeness is definitely established as cause of the polymelia. In addition animals with supernumerary hindlegs occurred in one of the cultures (G_{20}).*

Radical malformations could be observed in larvae ready to hatch and newly hatched. The simplest ones are the symmetrical double formations. The doubling begins without exception at the front end. In one case, which is shown in Fig. 1 of the plate** it reached close to the top of the tail on the dorsal side. In other more numerous cases, only the front part of the head is doubled. Frequently one of the twin parts is smaller than the other. It is then forced to one side by the stronger one (Fig. 2). In these cases the difference in size appears to have existed already in the first rudimentary form. In the further development it can easily be observed that the smaller part relatively falls behind, is pushed aside and finally is grown over by the larger one. Such forms constitute the transition from the double formations to the parasitic malformation.

The difference in size between the parts of the double formation is as a rule considerable from the beginning. The parasite then often lies as Figs. 3 and 4 show. The front part is grown together with the body of the carrier and deflected from it at a right or obtuse angle. Back and tail extend laterally along the tail of the carrier. Such parasites are much reduced. Mouth and suckers are rarely present, but gill rudiments are more frequent. In such a monster raised further, active respiratory movements could be observed in the parasite about ten days later.

It happens occasionally that several parasites are attached to the same carrier, but are more reduced, the farther back they are attached.

Finally, there are cases where the parasite is a complete amorphus, consisting only of shapeless tissue proliferations (Fig. 5 and 7)

With regard to the question of the causal genesis of these malformations, I have already in the above mentioned *Festschrift* demonstrated that it cannot possibly be a matter of super-regeneration process, and only because no "regeneration" takes place. The involvement of wounds, fractures, constrictions or similar factors can be excluded with certainty.

In order to clarify the problem further, I have this year for the third time initiated overripeness experiments and have directed my attention primarily toward the first development processes of the egg. The overripe egg is to be regarded as one of the processes of dying. That also follows from /35 the experiment with female Y. When she was selected for the experiment she

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*This spring (1922) I again obtained from overripe eggs a large number of frogs with supernumerary arms and legs.
**The illustrations were drawn by Miss Frieda Böhning.

was in the act of copulating. About 300 eggs were removed surgically from one uterus and were inseminated artificially. From these about 280 larvae developed; thus the mortality of eggs and embryos amounted to only 7%. Four days later more than 450 eggs were removed in the same way. Of these 147 or 33% died. Of the rest 103 or 23% cripples had to be fixed, and only 208 hatched larvae which were mostly afflicted with malformations could be raised further. 162 animals reached metamorphosis. As the female finally was killed 6 days after the first operation, it was found that all eggs still remaining in the uteri were dead.

The uterine egg is therefore no resting cell as much as one might get this impression with regard to its nucleus, which remains for days at the stage of the second central spindle metaphase. Certain processes of a chemical nature take place therein, which, in the subsequent phases, manifest themselves as pre-ripeness, ripeness, overripeness and degeneration.

The question arises concerning the nature of these processes and their localization in the nucleus or in the plasma. Microscopically changes can be detected in both locations. For the majority of the malformations only the plasmatic changes appear to come under consideration as causes. As suitable investigations are not available, it may be advisable for the present to refrain from all conjecture about the chemical nature of the ripening process. (This is much easier here) because, after all, it acts only indirectly as a teratogenic factor. We learn about the direct causes for the above described malformations however by the study of segmentation, gastrulation and neurula formation in the overripe egg.

In the following I limit myself again to the description of the circumstances that can be perceived by observation of the living object. Investigations according to series of sections will be reported in a detailed paper.

The eggs of the normally ripe series Y developed in typical manner. The first two segmental planes intersect each other at right angles and stand vertically, the third lies horizontally. The latter separate four animal cells of the upper pole of the egg from four vegetative cells of the lower pole. The former are somewhat smaller than the latter and can therefore be designated micromers. They are nevertheless so large that by polar observa- <u>/36</u> tion the macromers are nearly hidden by them (Cf. adjacent Fig. A, stage a). After the fourth segmentation the eight animal cells hide the eight vegetative ones still more completely (Fig. A, stage b)
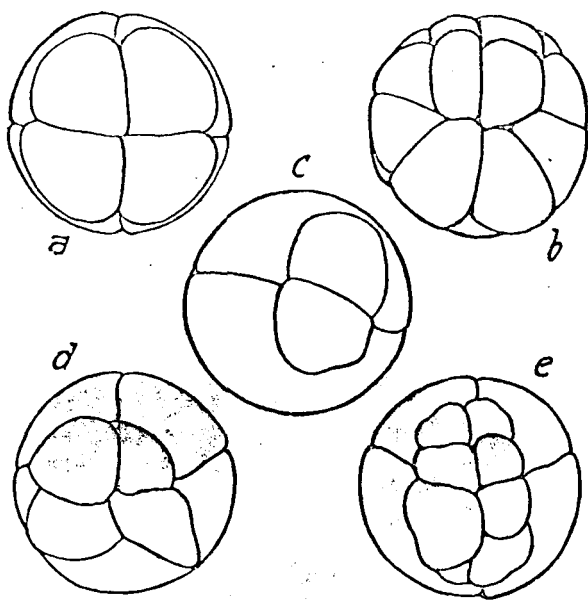
Fig A. Segmentation Stages. a, b normal eight and sixteen stages (Y'); d, e corresponding overripe stages (Y"); c, four cell stage of very overripe eggs (Y").

The eggs of the overripe series show strong deviations in comparison with this typical segmentation process. If we begin with the most insignificant modification, we see that the first two divisions proceed quite normally. The third horizontal cleavage, however, cuts through very high. Consequently, the micromers of the overripe eggs are much smaller than normal (Fig.A,d,e) With eggs that are pronouncedly altered, the sequence of the cleavage planes is changed. The first cleavage is still vertical, but the second is horizontal, so that only two micromers are cut off (Fig A,c). Further development turns out quite complicatedly. The macromers first cut off a second pair of macromers before the first splits off. In the case of extremely overripe eggs the cleavage is often first limited to the animal portion of the egg, while the meridional /37 cleavages proceed very slowly toward the vegetative pole. Finally, there are cases which interest us less here, however, in which the cleavage proceeds quite pathologically as so-called baroque cleavage, as described by Oskar Hertwig [2]. Perhaps polysperimia occasionally also plays a part. That may be the case when multipoled mitotic spindles occur and consequently the first segmentation produces four or more blastomers.

These characteristic changes of the segmentation process can, in short, be regarded as a progressive increase in the inertia of the plasma until it finally becomes incapable of segmentation.

In the morulation and gastrulation stages especially, all kinds of irregularities due to overripeness damage can be observed. It can almost always be determined that the development of the vegetative part is highly retarded. But often whole sectors are also found that can extend to the upper pole, (or also, irregularly delimited parts of the animal side remain without cleavage for a long time). (Fig. B) When they then finally do divide, cell heaps arise which form only loose tissue plugs between the apparently normal morular epidermis. Later they begin to flake off and are probably actively rejected by the adjacent sound tissue. Even at this stage the parts of the embryo that are capable of development can be divided into more or less separated areas.
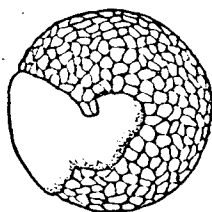
Fig. B   Fig. C
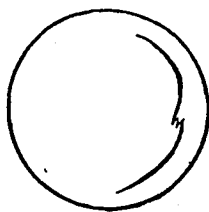
Fig. B   Morula with Sector Remaining without Cleavage (Y")

Fig. C   Double Blastomer Formation (Y")

Usually the morular cells are more rounded than normal, which indicates that they are only loosely connected to each other.

The processes of the gastrulation and the medullary tube formation are shown to be of the greatest teratogenic importance. In the gastrulation, conspicuous irregularities are noted with regard to the formation of the blastopore lip (Fig C) Instead of a slightly curved fold, long grooves appear which often are curved several times. This can probably be designated as a rudimentary multiple blastopore and obviously we have present here the earliest stage of the double malformation. The further development of such embryos proceeds quite slowly and many embryos die.

As O. Hertig has described (loc. cit.) spina bifida can result from incomplete gastrulation. Often the drawing together of the medulloblasts also is prevented by onflowing degenerated material. Actual double formations as described above did not appear any more at this stage.

The observations at the latest development stage of the overripe eggs consequently permit the recognition in the following two manifestations of the cause of the appearance of multiple formations:

1. The intercellular bonds are loosened and a tendency therefore exists for independent development of a cell or part of the embryo.
2. The separatist tendencies are further promoted by the fact that not all parts of the egg are damaged to the same degree by the overripeness. Consequently the embryo is often broken up into several viable parts by cords of degenerating tissue.

The immediate cause of the malformations due to overripeness therefore always consists in a disturbance or complete dissolution of the bonds and mutual control of the parts of the embryo. The isolated parts similarly to the blastomers or to embryo parts separated through tying off by Speman, Tornier and others. The greater a coherent part is, the stronger the tendency will generally show itself to be for formation of a complete embryo. Smaller pieces later come under the influence of the principal part, however. Their growth will be inhibited by the latter and in many cases complete resorption follows.

Let me mention only briefly in conclusion the cases which might be of greater interest for general pathology. In fact it sometimes happens that either the entire embryo consists only of a shapeless but strongly quivering heap of cells, or that such areas are scattered in an otherwise pronouncedly pathologically altered body (Plate V Fig. 6). In the latter case the pro-

liferations spread even farther out. A quite irregular growth starts, thick swellings sprout from it, and between them lie gaping fissures. First the growth of the embryos and larvae are severly retarded. The head mostly remains small and misshappen; no gills sprout forth, the tail remains short, and the body is much distended. Because of the strong quivering, the embryos rotate rapidly in their coats of jelly. As the degeneration of the tissues spreads even farther, such larvae are soon destroyed.

It appears to me that the overripeness experiments here briefly described contribute partly to the solution of certain very timely problems of human pathology. The causal genesis of multiple formations and teratisms in humans is still today shrouded in complete darkness. It is certain that only a very limited number is of a purely hereditary nature, and only a few not very characteristic cases can be attributed to entanglement with and attachment to the amnion. Yet the experimental factors which hitherto permitted the biologist to produce double formations at will do not come into consideration as direct causes, because the human embryo is completely protected as it were, during the critical stages of development, from mechanical and thermal disturbances; and, in addition, the tonic and chemical fluctuations of the environment are probably negligible. The overripeness experiments, whose causal connections of course are much more complicated than for example those of the tie-off experiments of Speman, derive their particular importance from the circumstance that here a factor is active which also can come into consideration as a cause of human malformation.

From our experiments interesting points of comparison with those on fishes, by Ch. Stockard, emerge. By reduction of the oxygen, segmentation was brought to a stop. When it was started again by renewed oxygen supply, accessory blastopore introversions were formed and double formations arose as here described. Stockard did not hesitate to utilize these results to explain the known normal polyembryony of the armadillo [3]. Certainly caution is always required in analogy conclusions from the yolk-rich eggs of fishes and amphibians to those of mammals. The overripeness malformations with their continuous series of anterior symmetrical duplicates up to the amorphus and to ulceration nevertheless show so far-reaching a similarity to human malformations that the conclusion of similar cause simply suggests itself. It is hoped that it will be possible by experiments on mammals to obtain definitive clarification.
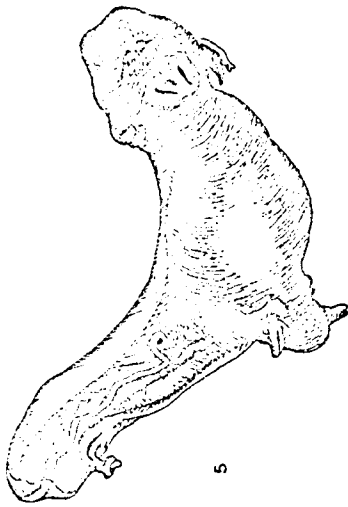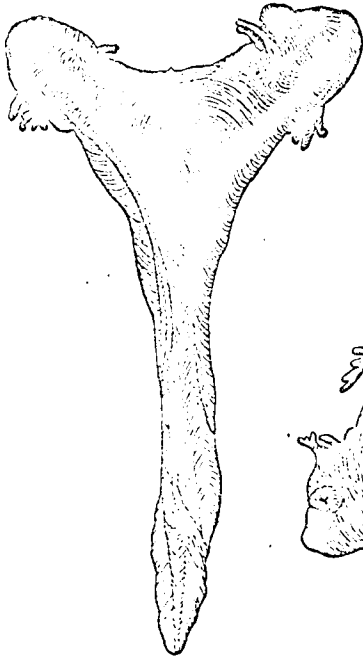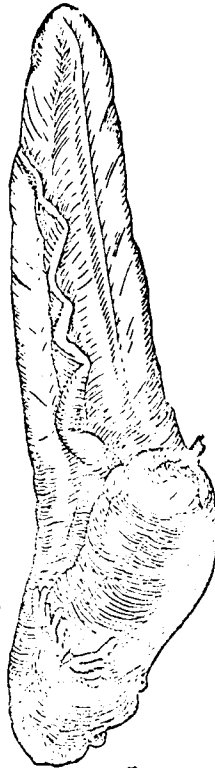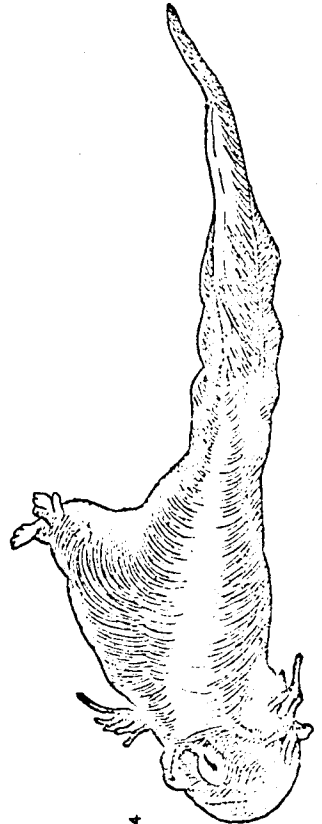
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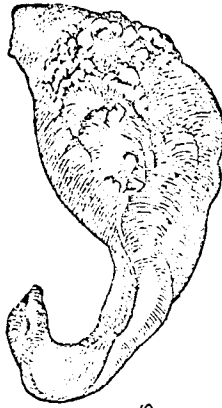
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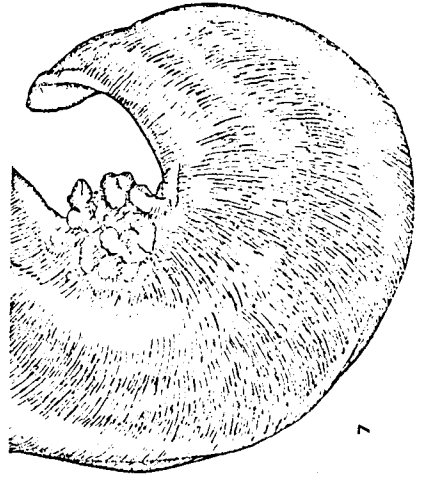
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Explanations to Plate V

Fig. 1. Symmetrical Double Formation, Dorsal View (No. 1, H_{20}).

Fig. 2. Asymmetrical Double Formation; The displaced right side head is imperfectly developed, with rudimentary gills and an odd spinning gland. Ventral View (No. 16, H_{20}).

Fig. 3,4 Lateral and Ventral View of a Larva with a Parasite (No. 3, H_{20}).

Fig. 5. Larva with Several Parasites of Different Forms (No. 19, H_{20}).

Fig. 6. Ventral View of a Larva with Reduced Head and Abscesslike Tissue Degeneration Mainly in the Chest Region (No. 20, H_{20}).

Fig. 7. Amorphous Proliferation on the Tail of an Otherwise Normal Larva (No. 64, H_{20}).

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